Cross-talk between cancer-initiating cells and immune cells: considerations for combination therapies

Amy S. Codd, Saly Al-Taei, Zsuzsanna Tabi

Division of Cancer and Genetics, School of Medicine, Cardiff University, Cardiff, CF14 2TL, UK

Correspondence to: Dr. Zsuzsanna Tabi. Division of Cancer & Genetics, School of Medicine, Cardiff University, Velindre Cancer Centre, Cardiff, CF14 2TL, UK. Email: tabiz@cardiff.ac.uk.

Submitted Sep 20, 2016. Accepted for publication Sep 25, 2016.

doi: 10.21037/atm.2016.10.30

View this article at: http://dx.doi.org/10.21037/atm.2016.10.30

Cancer initiating cells (CIC) or cancer stem-like cells (CSC) represent a small, distinct population of cancer cells best characterized by their high tumorigenicity. They undergo asymmetric cell division that results in repopulation of the bulk of the tumor and self-renewal. CICs are likely responsible for treatment failure and tumor recurrence, as they are highly resistant to traditional therapies, such as chemo- and radiotherapy (1). This resistance is partly due to their proliferative quiescence and increased anti-apoptotic features. Immune targeting is an emerging alternative approach, which may override these resistance mechanisms. However, relatively little is known about the interaction between CICs and immune cells that have the capacity to recognise and destroy not only the bulk of the tumor but also CICs.

In squamous cell carcinoma of the head and neck (SCCHN), CICs have been identified as a subpopulation of CD44+ cells. In a recent publication (2), Lee *et al.* carried out a comparative study between the immune behaviour of CD44+ and CD44– cells in SCCHN. The CD44 marker alone is not sufficient to define the CIC population without other markers. ALDH bright cells e.g., represent a subpopulation of CD44+ cells displaying enhanced clonogenic, tumorigenic capacity and radioresistance (3). Nevertheless, the study (2) provides some novel information about potential enhanced immunosuppressive features of the CIC-containing population of tumor cells. The key observation is the preferential expression of the immune checkpoint molecule programmed death-ligand 1 (PD-L1) on CD44+ *vs.* CD44– SCCHN cells. PD-L1 binds to PD-1 on T cells, inhibiting their function. In this study, IFNγ secretion by autologous CD8+ tumor-infiltrating lymphocytes (TIL) was inhibited by CD44+ cells. In another study, using a SCCHN cell line, CD44+ cells have also been shown to produce more TGFβ and IL-8, but not TNFα and IL-6, compared to that by CD44– cells (4). These CD44+ cells also inhibited T cell proliferation more strongly than CD44– tumor cells. However, neither study addressed the question of how susceptible these tumor cells are to T cell killing. Highly differentiated cytotoxic T lymphocytes (CTL) can be fairly resistant to immunosuppression, thus the effect of CD44+ tumor cells on CTL effector function would have been a more relevant T cell function to study. Indeed, it has been shown that SCCHN CICs, grown in 3D spheroids and identified by elevated ALDH levels, Nanog, Sox and Oct3/4 expression, are more susceptible to allo-specific T cell killing than tumor cells that are lower or negative for these markers (5).

Successful targeting of CICs by antigen-specific T cells in other types of cancer has also been demonstrated. Novel/mutated, overexpressed, oncofoetal, post-translationally altered and cancer testis (CT) are types of tumor-associated antigens T cells recognize (6). In the SCCHN study, discussed above (2), the cognate antigens were not identified so the question remains whether the proportions of tumor cells were comparable in the CD44+ and CD44– populations and if they differed in their antigen profile. As antibody blockade of the PD-1/PD-L1 interaction only partially reversed the inhibitory effect on autologous TIL-derived T cells, this possibility cannot be excluded. Biomarkers of CICs (e.g., ALDH1 or CD133) are also being investigated as immune targets (7). ALDH1A1-specific CD8+ T cells were shown to kill ALDH (bright) SCCHN CICs *in vitro* and T cell transfer resulted in better tumor control *in vivo* (8). However, the variety and reliability of markers associated with CICs, along with the potential for damaging healthy stem cells, expressing stem-related markers, may detract from this approach. CT antigens have been found to be frequently expressed in SCCHN (9), with e.g., MAGE A3/6 being an independent prognostic factor for tumor recurrence. Thus, CTL or monoclonal antibodies targeting CT antigens may be effective against both CICs and non-CICs in SCCHN. Interestingly, some antigens specific to CICs (e.g., BORIS, DNAJB8) have been reported to be more immunogenic than "shared" antigens (10,11). On the other hand, CD271+ melanoma CICs frequently lack the expression of melanoma tumor antigens TYR, MART1 and MAGE C1/C2 (12). This means that targeting these antigens would not affect CIC survival.

The antigen presentation ability of CICs, enabling interactions between cytotoxic T cells and peptide-HLA/MHC complexes, is also under debate. Low HLA class I expression and defective antigen processing machinery have previously been reported in CICs, in common with healthy stem cells (13). Contrastingly, Lee *et al*. reported that CD44+ and CD44− cells expressed comparable, high levels of HLA-ABC in the presence or absence of IFNγ (2). This suggests the differences in T cell modulation by CD44+ and CD44− cells may not be associated with their antigen processing ability. It may be useful to extend this line of investigation by e.g., transfecting CD44+ and CD44− cells with a surrogate antigen and determine the cognate T cell response.

A successful adaptive immune response, in which effector T cells eliminate CICs in an overt tumor and memory T cells persist to eliminate emerging CICs, would be crucial to achieve long-term tumor control. However, the interaction between tumor-infiltrating immune cells and CICs is still poorly understood as most studies are conducted *in vitro* rather than *in situ*. Whilst numerous immunosuppressive mechanisms have been attributed to CICs (14), most of these mechanisms are not unique to this population of cancer cells. There is a possibility that CICs are immune-protected rather than being immunosuppressive in their own right. CICs reside within cellular 'niches', which aid their immune protection and survival. These may include enhanced chemoattraction of tumor-associated macrophages (TAM) by CICs and skewing towards immunosuppressive M2-type macrophages (15,16). In SCCHN, self-renewal of CICs, identified by CD44 and ALDH expression, has been promoted by endothelial cell-secreted factors (17). Furthermore, 80% of these CICs were found in close proximity to blood vessels in the tumor tissue. Elimination of tumor-associated endothelial cells significantly reduces the proportion of CICs in xenografts (17). These findings indicate that endothelial cell-initiated signaling can enhance the survival and self-renewal of CICs, providing a unique, protective microenvironment for these cells.

The observation that PD-L1 is expressed on some CD44+ cells (but not on CD44− cells) in SCCHN (2) seems to confirm the theory that CICs are associated with enhanced immune protection. PD-1/PD-L1 inhibition as a therapeutic target in SCCHN is being tested in numerous clinical trials both alone and in combination (18). PD-L1 can also be expressed on infiltrating immune cells, as an effect of e.g. stromal factors, as shown by Spary *et al*. (19). However, correlation between PD-L1 expression on tumor cells or immune infiltrates and response to therapy has not been confirmed (20). Nevertheless, PD-L1 targeting maybe crucial for releasing the full potential of tumor antigen-specific effector T cells to target not only PD-L1+ differentiated tumor cells but also CICs and immunosuppressive TAMs.

As a conclusion, further studies with more precise identification of CICs and better definition of both their immunosuppressive nature and susceptibility to immune attack are clearly needed. Future treatment combinations may be improved by simultaneous targeting of the microenvironment, immune checkpoints, CICs and the bulk of the tumor in order to deliver direct and indirect hits both for tumor destruction and protection from recurrence.

Acknowledgements

AS Codd is supported by a Tenovus Cancer Care PhD scholarship.

Footnote

Provenance: This is a Guest Commentary commissioned by Section Editor Hongcheng Zhu, MD, PhD (Department of Radiation Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China).

Conflicts of Interest: The authors have no conflicts of interest to declare.

Comment on: Lee Y, Shin JH, Longmire M, *et al*. CD44+ Cells in Head and Neck Squamous Cell Carcinoma Suppress T-Cell-Mediated Immunity by Selective Constitutive and Inducible Expression of PD-L1. Clin Cancer Res 2016;22:3571-81.

References

1. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 2008;8:755-68.
2. Lee Y, Shin JH, Longmire M, et al. CD44+ Cells in Head and Neck Squamous Cell Carcinoma Suppress T-Cell-Mediated Immunity by Selective Constitutive and Inducible Expression of PD-L1. Clin Cancer Res 2016;22:3571-81.
3. Oh SY, Kang HJ, Kim YS, et al. CD44-negative cells in head and neck squamous carcinoma also have stem-cell like traits. Eur J Cancer 2013;49:272-80.
4. Chikamatsu K, Takahashi G, Sakakura K, et al. Immunoregulatory properties of CD44+ cancer stem-like cells in squamous cell carcinoma of the head and neck. Head Neck 2011;33:208-15.
5. Liao T, Kaufmann AM, Qian X, et al. Susceptibility to cytotoxic T cell lysis of cancer stem cells derived from cervical and head and neck tumor cell lines. J Cancer Res Clin Oncol 2013;139:159-70.
6. Yamada R, Takahashi A, Torigoe T, et al. Preferential expression of cancer/testis genes in cancer stem-like cells: proposal of a novel sub-category, cancer/testis/stem gene. Tissue Antigens 2013;81:428-34.
7. Pan Q, Li Q, Liu S, et al. Concise Review: Targeting Cancer Stem Cells Using Immunologic Approaches. Stem Cells 2015;33:2085-92.
8. Visus C, Wang Y, Lozano-Leon A, et al. Targeting ALDH(bright) human carcinoma-initiating cells with ALDH1A1-specific CD8+ T cells. Clin Cancer Res 2011;17:6174-84.
9. Zamunér FT, Karia BT, de Oliveira CZ, et al. A Comprehensive Expression Analysis of Cancer Testis Antigens in Head and Neck Squamous Cell Carcinoma Revels MAGEA3/6 as a Marker for Recurrence. Mol Cancer Ther 2015;14:828-34.
10. Asano T, Hirohashi Y, Torigoe T, et al. Brother of the regulator of the imprinted site (BORIS) variant subfamily 6 is involved in cervical cancer stemness and can be a target of immunotherapy. Oncotarget 2016;7:11223-37.
11. Morita R, Nishizawa S, Torigoe T, et al. Heat shock protein DNAJB8 is a novel target for immunotherapy of colon cancer-initiating cells. Cancer Sci 2014;105:389-95.
12. Boiko AD, Razorenova OV, van de Rijn M, et al. Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. Nature 2010;466:133-7.
13. López-Albaitero A, Nayak JV, Ogino T, et al. Role of antigen-processing machinery in the in vitro resistance of squamous cell carcinoma of the head and neck cells to recognition by CTL. J Immunol 2006;176:3402-9.
14. Maccalli C, Volontè A, Cimminiello C, et al. Immunology of cancer stem cells in solid tumours. A review. Eur J Cancer 2014;50:649-55.
15. Silver DJ, Sinyuk M, Vogelbaum MA, et al. The intersection of cancer, cancer stem cells, and the immune system: therapeutic opportunities. Neuro Oncol 2016;18:153-9.
16. Sainz B Jr, Carron E, Vallespinós M, et al. Cancer Stem Cells and Macrophages: Implications in Tumor Biology and Therapeutic Strategies. Mediators Inflamm 2016;2016:9012369.
17. Krishnamurthy S, Dong Z, Vodopyanov D, et al. Endothelial cell-initiated signaling promotes the survival and self-renewal of cancer stem cells. Cancer Res 2010;70:9969-78.
18. Honeychurch J, Cheadle EJ, Dovedi SJ, et al. Immuno-regulatory antibodies for the treatment of cancer. Expert Opin Biol Ther 2015;15:787-801.
19. Spary LK, Salimu J, Webber JP, et al. Tumor stroma-derived factors skew monocyte to dendritic cell differentiation toward a suppressive CD14+ PD-L1+ phenotype in prostate cancer. Oncoimmunology 2014;3:e955331.
20. Sponaas AM, Moharrami NN, Feyzi E, et al. PDL1 Expression on Plasma and Dendritic Cells in Myeloma Bone Marrow Suggests Benefit of Targeted anti PD1-PDL1 Therapy. PLoS One 2015;10:e0139867.

Cite this article as: Codd AS, Al-Taei S, Tabi Z. Cross-talk between cancer-initiating cells and immune cells: considerations for combination therapies. Ann Transl Med 2016. doi: 10.21037/atm.2016.10.30